Chromosome studies of three families of pelagic heteropod molluscs (Atlantidae, Carinariidae, and Pterotracheidae) from Hawaiian waters

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Abstract: Chromosome number and morphology were studied in gonadal tissue of 11 species of Atlantidae, 2 species of Carinariidae, and 3 species of Pterotracheidae, using an air-drying technique and Giemsa staining. In the Atlantidae the diploid chromosome number was the same in males and females and there were no heteromorphic chromosomes. The diploid chromosome number in nine species of *Atlanta* was 30 and the majority of chromosome pairs were metacentric and submetacentric. In *Protatlanta souleyeti* the diploid number was 28, and included five metacentric, six submetacentric, and three subtelocentric chromosome pairs. *Oxygyrus keraudreni* had a diploid chromosome number of 32, with 10 metacentric and 6 submetacentric chromosome pairs. A striking difference between the Atlantidae and the Carinariidae and Pterotracheidae was the presence of heteromorphic sex chromosomes in the latter two families. Male *Pterosoma planum* ($2n = 32$) had simple XY sex chromosomes, but males of *Carinaria japonica* ($2n = 33$), *Pterotrachea scutata* ($2n = 33$), *Pterotrachea hippocampus* ($2n = 31$), and *Firoloida desmaresti* ($2n = 31$) showed three heteromorphic chromosomes, suggesting a multiple sex-determining mechanism, X_1X_2Y. The locations of the female sex chromosomes in the karyotypes of female *Pterotrachea hippocampus* ($2n = 32$) and *Firoloida desmaresti* ($2n = 32$) were tentatively proposed. Cytogenetic features observed among the three families are supportive of previous interpretations of evolutionary trends in the Heteropoda based on morphology, i.e., that the Atlantidae are the most primitive family and gave rise to the Carinariidae and Pterotracheidae.

Résumé : Le nombre et la morphologie des chromosomes de 11 espèces d'Atlantidae, deux espèces de Carinariidae et trois espèces de Pterotracheidae, ont été étudiés à partir de tissu gonadique par une méthode de suspension cellulaire et coloration au Giemsa. Parmi les Atlantidae, les mâles et femelles montrent un même nombre chromosomique diploïde et l'absence de chromosomes hétéromorphiques. *Protatlanta souleyeti* présente le nombre diploïde le plus bas, 28 (cinq paires de chromosomes métacentriques, six submetacentriques et trois subtelocentriques), *Oxygyrus keraudreni* a le plus grand nombre diploïde (32) (10 paires métacentriques et 6 submétacentriques) et neuf espèces d'Atlanta montrent un même nombre diploïde de 30 avec des caryotypes constitués majoritairement de chromosomes métacentriques et submétacentriques. La présence de chromosomes sexuels hétéromorphes chez les Carinariidae et Pterotracheidae marque leur séparation d'avec les Atlantidae. *Pterosoma planum* ($2n = 32$ chez les mâles et les femelles) montre des chromosomes sexuels de type XY tandis que les mâles de *Carinaria japonica* ($2n = 33$), *Pterotrachea scututa* ($2n = 33$), *Pterotrachea hippocampus* ($2n = 31$) et *Firoloida desmaresti* ($2n = 31$) présentent trois chromosomes hétéromorphiques suggérant un déterminisme sexuel de type multiple X_1X_2Y. Les femelles de *Pterotrachea hippocampus* ($2n = 32$) et *Firoloida desmaresti* ($2n = 32$) ont été étudiées et la position de leurs chromosomes sexuels dans les caryotypes a été suggérée. Les critères caryologiques, observés dans l'ensemble des Hétéropodes, reflètent leurs traits évolutifs d'adaptation progressive à la vie pélagique depuis les formes primitives, telles les Atlantidae, jusqu'aux formes les plus spécialisées, les Carinariidae et les Pterotracheidae.

Introduction

The heteropods are a group of gastropod molluscs that live in the open ocean and exhibit morphological and reproductive adaptations to their pelagic life-style (Lalli and Gilmer 1989). They comprise a small group of taenioglossate mesogastropods consisting of about 28 species belonging to three families. The Atlantidae is considered to be the most primitive family, from which the Carinariidae and Pterotracheidae were derived; the latter two families display progressive adaptations to a pelagic life-style. Of the three families, evolutionary relationships have been hypothesized only within the Atlantidae (Richter 1973). Another group of pelagic gastropods, the thecosome pteropods, also exhibit morphological adaptations to the pelagic environment (Lalli and Gilmer 1989), which Thiriot-Quiévreux (1988) related to chromosomal diversity, i.e., an increase in diploid chromosome number and an increase in the proportion of subtelocentric– telocentric chromosomes from the primitive to the most specialized species.

Unlike those for thecosome pteropods, chromosomal data for heteropods are very limited. Boveri (1890) reported 32

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Table 1. Summary of karyological data for species of Heteropoda from Hawaiian waters.

Family and species	No. of animals		No. of metaphases scored	No. of karyotypes	Diploid chromosome no.	Morphological type (no. of chromosome pairs)			No. of heteromorphic sex chromosomes
	Males	Females				*m*	*sm*	*st*	
Atlantidae									
Oxygyrus keraudreni	1	5	25	8*	32	10	6		
Protatlanta souleyeti	2	2	17	6*	28	5	6	3	
Atlanta lesueuri	2	4	30	10*	30	5	10		
Atlanta oligogyra	5	4	25	4*	30	9	5	1	
Atlanta peroni	3	5	18	8*	30	7	8		
Atlanta plana	3	8	34	10*	30	9	6		
Atlanta fusca	1		9	4*	30	11	4		
Atlanta turriculata	8	1	25	7*	30	4	11		
Atlanta meteori	5	3	28	8*	30	7	7	1	
Atlanta tokiokai		2	4	3*	30	6	6	3	
Atlanta inflata	1		3	1	30				
Carinariidae									
Carinaria japonica	1		10	2 (1*)	33	9	6		3
Pterosoma planum	1		15	3*	32	5	5	5	2
		2	16	7*	32	5	6	5	
Pterotracheidae									
Pterotrachea scutata	1		18	8 (2*)	33	7	8		3
Pterotrachea hippocampus	6		19	5 (1*)	31	6		8	3
		7	14	3 (1*)	32	7		9	
Firoloida desmaresti	3		6	3 (1*)	31	8		6	3
		4	8	4 (1*)	32	10		6	

Note: *m*, metacentric; *sm*, submetacentric; *st*, subtelocentric.
*Number of karyotypes with chromosome measurements.

chromosomes in females of *Carinaria mediterranea* Blainville (= *C. lamarcki* Peron and Lesueur) and *Pterotrachea mutica* Smith (= *P. hippocampus* Philippi). Recently, three species of Pterotracheidae from Mediterranean waters, *P. hippocampus, P. coronata* Forskal, and *Firoloida desmaresti* Lesueur, were studied by Thiriot-Quiévreux (1990). Females of *P. coronata* and *F. desmaresti* had a diploid number of 32. However, the diploid number for males belonging to these three species was 31, which was explained by an unusual mechanism of sex determination. A karyological study of *P. hippocampus* from the Gulf of Palermo by Vitturi et al. (1993) confirmed the above diploid chromosome numbers for this species and the presence of a multiple sex chromosome system, designated X_1X_2Y in males and $X_1X_1X_2X_2$ in females.

In this paper, chromosome number and morphology are described for 16 species of Heteropoda from Hawaiian waters, including 11 of the 13 species of Atlantidae (Seapy 1990), 2 of the 4 species of Carinariidae (Seapy 1987), and 3 of the 4 species of Pterotracheidae (Seapy 1985, 1987). We also have attempted to relate similarities and differences among the karyotypes to evolutionary relationships within the Heteropoda.

Materials and methods

Specimens of heteropods belonging to Atlantidae (11 species), Carinariidae (2 species) and Pterotracheidae (3 species) were collected by oblique tows in the upper 100 m from waters off the islands of Kaua'i and Nihoa using a 1.6-m plankton net aboard the R/V *Hokusei Maru*, Hokkaido University (6–13 February 1990), and off the islands of Moloka'i, Lana'i, and Maui using a 1.0-m plankton net aboard the R/V *MoanaWave*, University of Hawaii (8–17 January 1992).

Initial phases of chromosome preparation were completed as soon as possible after the animals were collected and sorted from the plankton samples aboard ship. Specimens at a stage of gametogenesis that would provide mitotic metaphases were incubated for 2 h in 0.005% colchicine in seawater and were then transfered to 0.9% sodium citrate in distilled water for 30 min. For the Atlantidae, shells were gently broken in the hypotonic sodium citrate solution to allow it to penetrate. For the Carinariidae and Pterotracheidae, the visceral nucleus was dissected out in the sodium citrate solution. Fixation was in a freshly prepared mixture of absolute ethanol and acetic acid (3:1). Specimens were kept refrigerated until they could be processed in the laboratory.

Slides were made from gonadal tissue, using an air-drying technique (Thiriot-Quiévreux and Ayraud 1982), and were then stained with 4% Giemsa, pH 6.8. Chromosome analyses were made according to Thiriot-Quiévreux (1988) and Insua et al. (1994). Terminology relating to centromere position follows that of Levan et al. (1964). The number of cells measured varied among the species, depending upon the number of well-spread metaphases available. Table 1 summarizes the data for each species studied. The karyotypes are shown in the figures; photomicrographs of chromosome pairs are arranged in rows of the same morphological type, i.e., metacentric, submetacentric or subtelocentric.

Results

Atlantidae

Males and females were studied and showed the same diploid chromosome numbers, with no heteromorphic chromosomes.

Fig. 1. Karyotypes of Atlantidae. (A) Male *Oxygyrus keraudreni* ($2n = 32$). (B) Juvenile *Protatlanta souleyeti* ($2n = 28$). (C) Male *Atlanta lesueuri* ($2n = 30$). (D) Male *Atlanta peroni* ($2n = 30$). (E) Male *Atlanta meteori* ($2n = 30$). Scale bar = 5 μm.

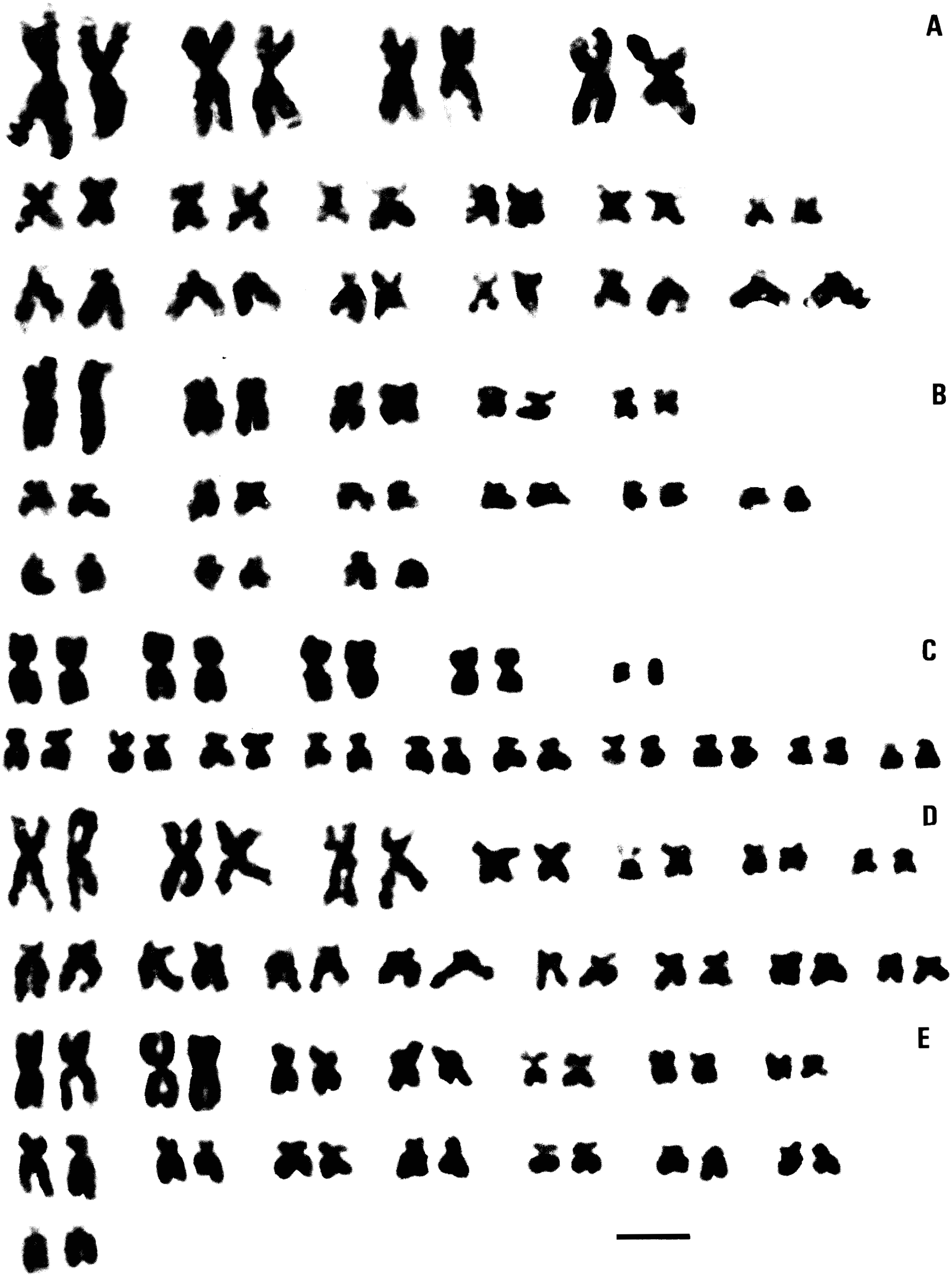


Fig. 2. Karyotypes of Carinariidae. (A) Male *Carinaria japonica* with three heteromorphic chromosomes (arrows) ($2n = 33$). (B) Male *Pterosoma planum* with two heteromorphic chromosomes (arrows) ($2n = 32$). (C) Female *Pterosoma planum* ($2n = 32$). Scale bar = 5 μm.

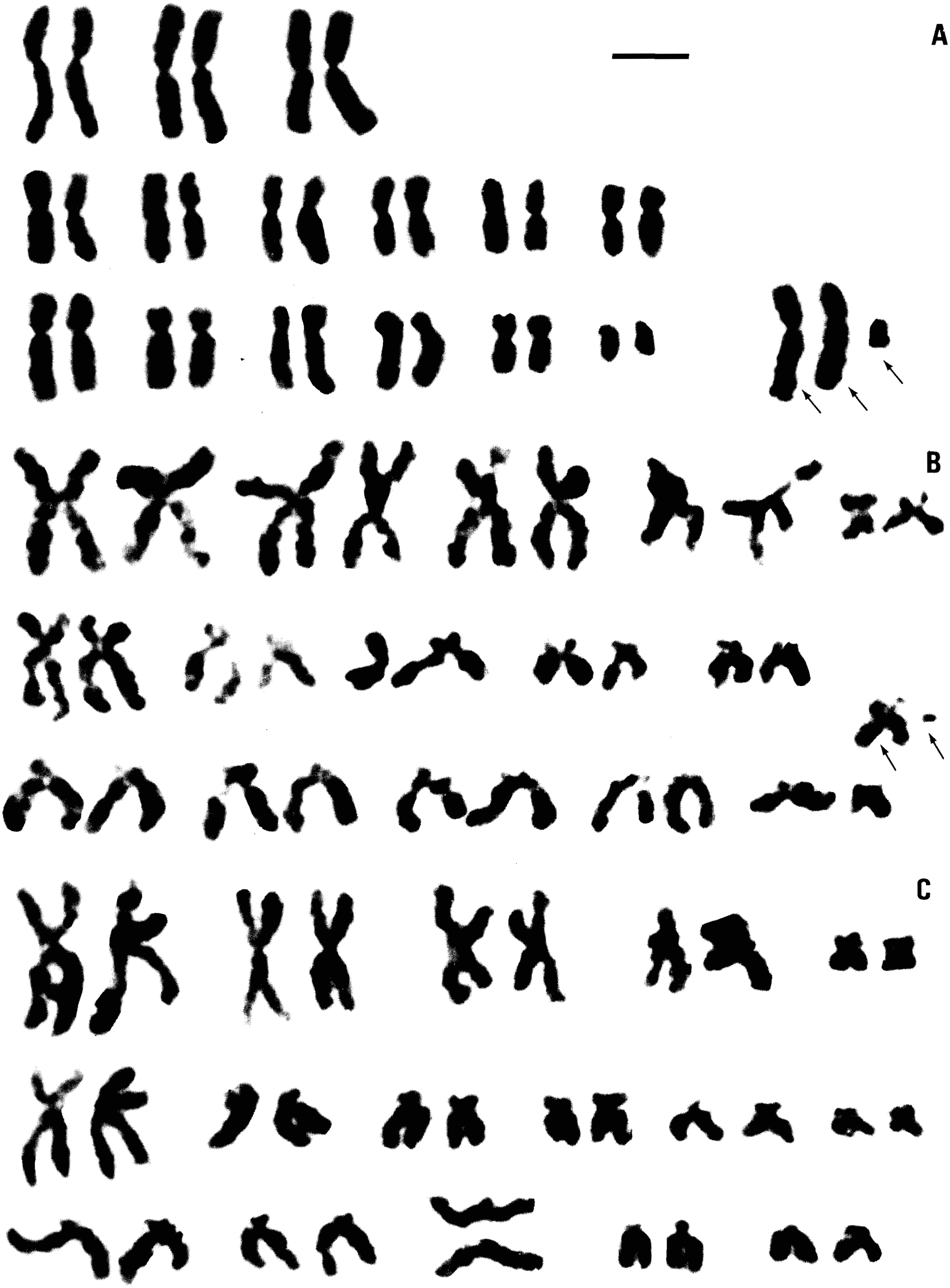
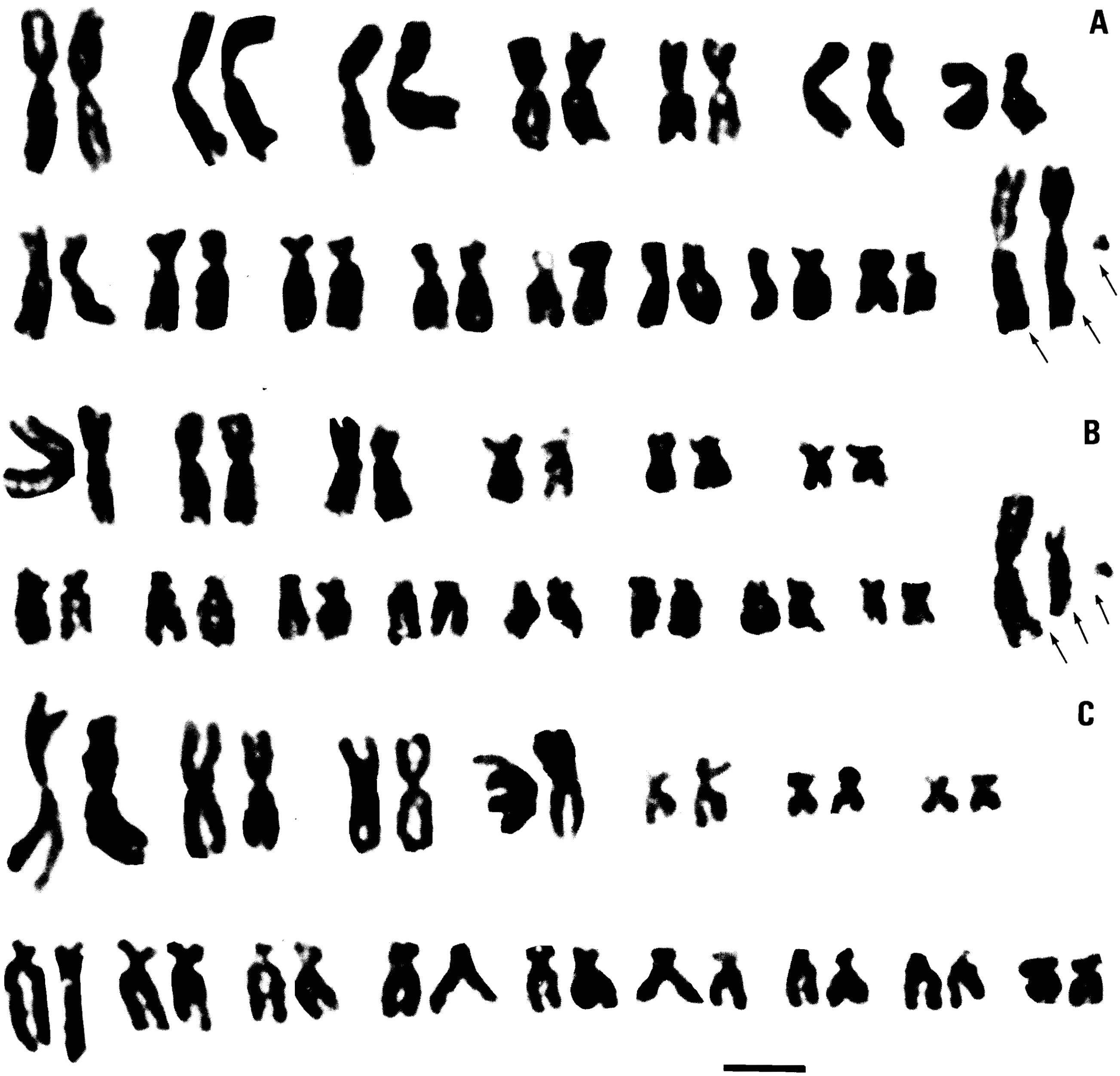


Fig. 3. Karyotypes of Pterotracheidae (*Pterotrachea* spp. (A) Male *P. scutata* with three heteromorphic chromosomes (arrows) ($2n$ = 33). (B) Male *P. hippocampus* with three heteromorphic chromosomes (arrows) ($2n$ = 31). (C) Female *P. hippocampus* ($2n$ = 32). Scale bar = 5 μm.



Oxygyrus keraudreni (Lesueur, 1817)
Mitotic metaphases showed a diploid number of 32. The karyotype (Fig. 1A) consisted of 10 metacentric and 6 submetacentric chromosome pairs.

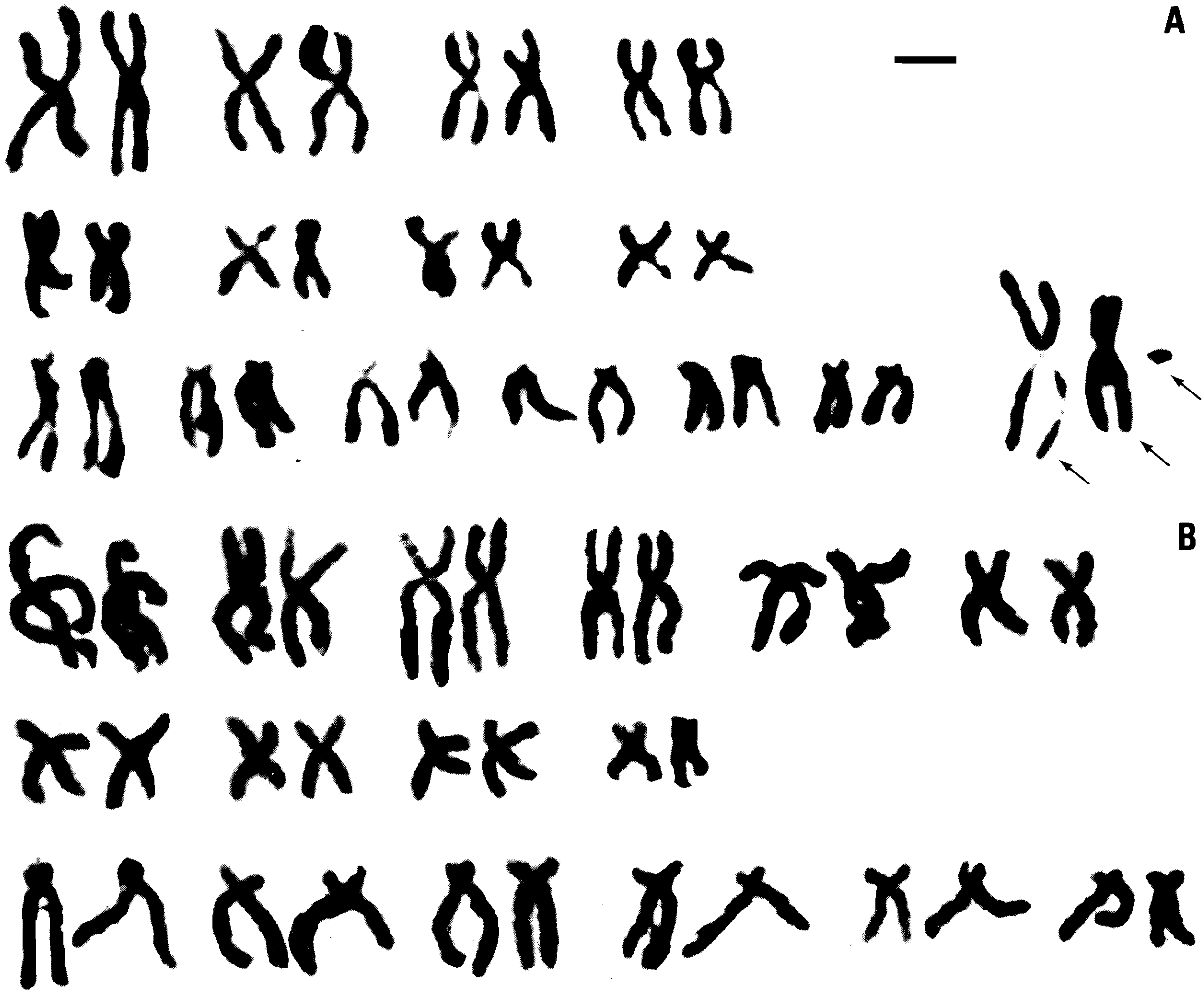
Protatlanta souleyti (Smith, 1888)
With a diploid number of 28, the karyotype (Fig. 1B) included five metacentric, six submetacentric, and three subtelocentric chromosome pairs. The first pair was much larger than the remaining pairs.

Atlanta spp.
Nine species were investigated, all showing a diploid number of 30. The numbers of metacentric, submetacentric, and subtelocentric chromosome pairs for the species are summarized in Table 1. The karyotypes showed a majority of metacentric and submetacentric chromosome pairs, e.g., in *A. lesueuri* (Fig. 1C) and *A. peroni* (Fig. 1D). Subtelocentric pairs occurred infrequently, e.g., in *A. meteori* (Fig. 1E).

Carinariidae

Carinaria japonica Okutani, 1955
Only one male was collected. Mitotic metaphases showed a diploid number of 33. Two karyotypes showed nine metacentric and six submetacentric chromosome pairs, and three unpaired and heteromorphic chromosomes, including a large metacentric chromosome, a large submetacentric chromosome, and a microchromosome (Fig. 2A).

Fig. 4. Karyotypes of Pterotracheidae: *Firoloida desmaresti*. (A) Male with three heteromorphic chromosomes (arrows) ($2n = 31$). (B) Female ($2n = 32$). Scale bar = 5 μm.



Pterosoma planum Lesson, 1827
Males and females showed the same diploid number, 32. The karyotype in males (Fig. 2B) included five metacentric, five submetacentric, five subtelocentric chromosome pairs and two heteromorphic chromosomes, one of which was submetacentric and the other was a microchromosome. The female karyotype (Fig. 2C) had five metacentric, six submetacentric and five subtelocentric chromosome pairs. Thus, the heteromorphic chromosomes in the males could represent an XY pair and the XX chromosomes in females would be one of the submetacentric chromosome pairs.

Pterotracheidae

Pterotrachea scutata Gegenbaur, 1855
All chromosome preparations for this species were obtained from a single male specimen, and 18 metaphases were scored in total. The male diploid number was 33. The karyotype (Fig. 3A) consisted of seven metacentric and eight submetacentric chromosome pairs and three heteromorphic chromosomes, including one large metacentric and one large submetacentric chromosome and a microchromosome.

Pterotrachea hippocampus Philippi, 1836
With a diploid number of 31, the male karyotype (Fig. 3B) showed six metacentric and eight subtelocentric chromosome pairs and three heteromorphic chromosomes, including one large metacentric and one large subtelocentric chromosome and a microchromosome. The heteromorphic chromosomes in the male may represent the multiple sex chromosomes designated X_1X_2Y by Vitturi et al. (1993). The female karyotype (Fig. 3C) had a diploid number of 32, with seven metacentric and nine subtelocentric chromosome pairs. The X_1X_1 sex chromosomes may be the first of the largest metacentric chromosome pairs and the X_2X_2 sex chromosomes may be the first of the largest subtelocentric chromosome pairs.

Firoloida desmaresti Lesueur, 1817
Male mitotic metaphases showed a diploid number of 31. The karyotype (Fig. 4A) included eight metacentric and six subtelocentric chromosome pairs and three heteromorphic chromosomes, including two large metacentric chromosomes with different centromere positions and a microchromosome.

With a diploid number of 32, the female karyotype (Fig. 4B) had 10 metacentric and 6 subtelocentric chromosomes.

In contrast to the X_1X_2Y male sex chromosomes, the X_1X_1 female sex chromosomes could be the largest metacentric pair and the X_2X_2 as the third (?) largest metacentric pair (Fig. 4B).

Discussion

The range in diploid chromosome numbers among the heteropods examined in this study (28−33) is comparable to diploid chromosome numbers recorded in other mesogastropod families, e.g., 31−34 in Hydrobiidae (Kitikoon 1982), 34 in Littorinidae (Vitturi et al. 1986), and 34 in Naticidae (Vitturi et al. 1982; Komatsu 1985).

Within the Atlantidae, all nine species of *Atlanta* had the same diploid chromosome number (30), although those of the monospecific genera *Protatlanta* and *Oxygyrus* were different; the former lower ($2n = 28$) and the latter higher ($2n = 32$). The atlantid chromosomes were mainly metacentric and submetacentric, which may be considered characteristic of stable karyotypes (sensu Nakamura 1985).

Richter (1973) hypothesized evolutionary relationships in the Atlantidae based on shell and soft part characteristics. He considered *Atlanta fusca* the most primitive living species. From *A. fusca* he derived the remaining species of *Atlanta* and a separate lineage leading to *P. souleyeti* (in which the calcareous keel is replaced by a conchiolin keel) and *O. keraudreni* (in which the larval shell is calcareous but the adult shell and keel are composed of conchiolin). He then derived the Carinariidae from the Atlantidae by shell reduction and the Pterotracheidae from the Carinariidae by shell loss. Our karyological results corroborate a separate lineage for *Protatlanta* and *Oxygyrus*, although differences in chromosome number and morphology may not indicate which genus is ancestral. Within the genus *Atlanta*, the different proportions of metacentric, submetacentric, and subtelocentric chromosomes reported here may reflect cytotaxonomic differences, but no patterns appear to be related to Richter's hypotheses.

A striking difference between the Atlantidae and the other two heteropod families concerns their sex chromosomes. Among the species of Carinariidae and Pterotracheidae examined here, only *P. planum* had male sex chromosomes of the XY type. Simple male sex-determining chromosomes of this type have been reported in other prosobranchs (Patterson 1969; Stern 1975; Kitikoon 1982; Thiriot-Quiévreux and Ayraud 1982; Vitturi et al. 1988). The multiple-chromosome mechanism of sex determination, reported in Mediterranean *P. hippocampus* by Vitturi et al. (1993) as X_1X_2Y in males and $X_1X_1X_2X_2$ in females, was also observed here in Hawaiian *P. hippocampus* and *F. desmaresti*. We tentatively propose the location of the sex chromosomes in the karyotypes of female *P. hippocampus* and *F. desmaresti*. Males of *C. japonica* and *P. scutata* also showed heteromorphic chromosomes, suggesting a similar sex-determining mechanism. Additional karyological investigations should be carried out to verify and possibly extend these results. Comparison of karyotypes between Hawaiian and Mediterranean populations of *P. hippocampus* and *F. desmaresti* (this study; Thiriot-Quiévreux 1990; Vitturi et al. 1993) reveals differences in the observed numbers of metacentric, submetacentric, or subtelocentric chromosome pairs. Because the number of cells measured was limited, however, we can not infer at present the existence of differences in the karyotypes of Hawaiian and Mediterranean populations of these cosmopolitan species. Clearly, additional specimens need to be examined before intraspecific karyological differences can be hypothesized.

In summary, the karyological features of heteropod species reported here highlight the primitive and stable character of the genus *Atlanta* in the Atlantidae. Conversely, morphologically differentiated sex chromosomes were found in the Carinariidae and Pterotracheidae. The simple sex mechanism in *P. planum* could be considered a plesiomorphic trait, while the multiple sex-determining mechanism in the other carinariids and in the pterotracheids would be apomorphic. Chromosomal evolutionary trends in the pelagic Thecosomata (Thiriot-Quiévreux 1988) and Heteropoda are related to progressive adaptation to a pelagic life-style, from primitive to derived species.

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